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(Research Article)

Synthesis, Characterization and Biological Evaluation of Some Mannich Schiff Base Derivatives of Substituted Benzimidazole

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ABSTRACT

2-(4-aminophenyl) benzimidazole were reacted with p-nitro benzaldehyde to form a Schiff base compound. A series of Mannich bases of this compound were synthesized by reacting them with formaldehyde and different secondary amines. TLC and Column chromatography is used for the isolation and separation of the mixture of the compound with the help of silica gel G and GF and visualize by iodine chamber. All the newly synthesized compounds have been characterized by the IR, ^{1}H NMR, and MASS spectral data by structural elucidation. All newly synthesized compounds were evaluated for antibacterial activity by Well diffusion methods and anti-inflammatory method by carreageenan rat paw edema method.

Key words: Benzimidazole derivatives, Mannich Schiff Bases, Antibacterial activity, Anti-inflammatory activity.

INTRODUCTION

Mannich Bases of wide variety of heterocyclic/non-heterocyclic nucleus have been revealed to posses cytotoxic, antibacterial, antifungal, anticonvulsant, anti-inflammatory and antimalarial activity[1]. Benzimidazole derivatives were reported to possess antibacterial[2], antifungal[3], anti-inflammatory[4], antiviral[5], antitubercular[6], antioxidant[7], antiprotozoal[8], antihelmintic[9], antihypertensive[10], antidiabetic[11] and antiulcer[12] activity. The presence of basic mannich side chain in a drug may overcome the water insolubility problem through the formation of hydrochlorides[13].Therefore it was thought that preparing Mannich base derivatives from 2-substituted benzimidazoles would probably result in compounds having high biological activities towards many diseases. In this present study2-(4-aminophenyl)-benzimidazole were treated with different substituted aromatic aldehydes to produce Schiff bases[14-16]. The Schiff bases were subjected to Mannich reactions with formaldehyde and different secondary amines to produce Mannich bases derivatives[17-19] were prepared as per scheme. The chemical structures of the synthesized compounds were confirmed by IR, ^{1}H-NMR, mass spectral and elemental analysis. The synthesized compounds were screened for antibacterial (*Bacillus subtilis, Escherichia coli, Staphylococcus aureus and Klebsiella pneumonia*) and anti-inflammatory activity by Carrageenen induced paw edema method.

MATERIAL AND METHODS

The melting points were taken in open capillary tube and are uncorrected. All reactions were followed by TLC, with detection by UV light and/or visualized by iodine vapors. Column chromatography was performed on silica gel(60-120 mesh, Merck). IR spectra were recorded on Perkin Elmer (4000-450cm^{-1}) FTIR spectrophotometer. ^{1}H-NMR spectra were recorded on a Spect-300 MHz spectrophotometer in $CDCl_3$. Mass spectra were recorded on +c ESI (100.00-1000.00) Spectrophotometer.

Synthesis of 2-(4-aminophenyl)-benzimidazole

Taken the equimolar quantities (0.01 mol) of o-phenylene diamine, para amino benzoic acid (0.01 mol) in 4N HCL (20 ml) was reflux for 45 min. The mixture is cool and filtered off. The product is recrystallized from absolute alcohol.This compound was obtained as a grayish blue crystals; yield 96%; mp 209^{0}C-211^{0}C.

Preparation of Schiff Bases

Taken a mixture of equimolar quantities (0.01 mol) of aromatic aldehyde and 2-(4-amino phenyl) benzimidazole and add a drop of acetic acid was refluxed for 45-60 min in 25 ml of ethanol. The reaction mixture was cooled and kept for 24 h. The crystals found was filtered and dried. The product was recrystallized from ethanol. This compound was obtained as yellow solid; yield 78%; mp 255^{0}C- 256^{0}C.

Synthesis of Mannich Bases

A slurry consisting of 0.01 mol of Schiff base, 10 ml of tetra hydro furan and 4 ml of formaldehyde (37%) was made. To this secondary amine (0.01 mol) was added drop wise with cooling and shaking. The reaction mixture was allowed to stand at room temperature for 1h with occasional shaking

then it was warmed on a steam bath for 15 min. At the end of the period the contents were cooled and the product obtained was recrystallized from petroleum ether.

Compound BG1

This compound was obtained as a yellow solid; Yield =97%, mp 264^{0}C-265^{0}C, Rf =0.81, IR (KBr) cm^{-1}: 3683.5, 3405.56, 3022.24, 1709.43, 1604.14, 1286.28, 850.81; NMR($CDCl_3$):10.2(1H,s,J=10.169), 8.4(3H,t,J=8.419), 8.1(3H,t,J=8.072), 7.3(1H,s,J=7.271), 1.2(1H,s,J=1.281); Mass: m/z 399.22.C_{23},H_{21},N_5,O_2(398.29): Calcd: C=69.17%; H=5.26%; N=17.53%; O=8.01%; Found: C=69.29%; H=5.27%; N=17.57%; O=8.03%.

Compound BG2

This compound was obtained as a pale yellow solid; Yield =82%, mp 278^{0}C-279^{0}C, Rf =0.79, IR (KBr) cm^{-1}: 3405.78, 3022.49, 1709.93, 1530.91, 1347.17, 1287.59, 768.92; NMR($CDCl_3$): 10.2(1H,s,J=10.167), 8.4(3H,t,J=8.419), 8.1(3H,t,J=8.098), 7.3(1H,s,J=7.267), 3.1(1H,s,J=3.132); 2.8(1H,s,J=2.745) 1.4(1H,s,J=1.418); Mass: m/z 427.25.C_{25},H_{25},N_5,O_2(428.37): Calcd: C=70.21%; H=5.85%; N=16.38%; O=7.48%; Found: C=70.03%; H=5.83%; N=16.34%; O=7.47%.

Compound BG3

This compound was obtained as a cream yellow solid; Yield =87%, mp 290^{0}C-291^{0}C, Rf =0.73, IR (KBr) cm^{-1}: 3408.63, 3021.97, 1709.14, 1285.6, 1217.98, 814.82, 769.95; NMR($CDCl_3$):10.2(1H,s,J=10.167), 8.4(3H,t,J=8.39), 7.2(1H,s,J=7.267), 3.3(1H,s,J=3.343), 1.2(1H,s,J=1.254); Mass: m/z 427.22. $C_{24}H_{21}N_5O_3$ (428.35): Calcd: C= 67.41%; H=4.91%; N=16.38%; O=11.23%; Found: C=67.23%; H=4.90%; N=16.34%; O=11.20%.

Compound BG4

This compound was obtained as a pale yellow solid; Yield =94%, mp 283^{0}C-284^{0}C, Rf =0.64, IR (KBr) cm^{-1}: 3412.53, 3023.87,2848.51, 1708.6, 1530.66, 1288.69, 770.35; NMR($CDCl_3$): 8.3(3H,t,J=8.389), 8.1(3H,t,J=8.105), 4.0(1H,s,J=4.035), 2.6(1H,s,J=2.659); Mass: m/z 440.25. C_{25},H_{24},N_6,O_2(442.42): Calcd: C=68.14%; H=5.45%; N=19.08%; O=7.26%; Found: C=67.80%; H=5.42%; N=18.98%; O=7.23%.

Compound BG5

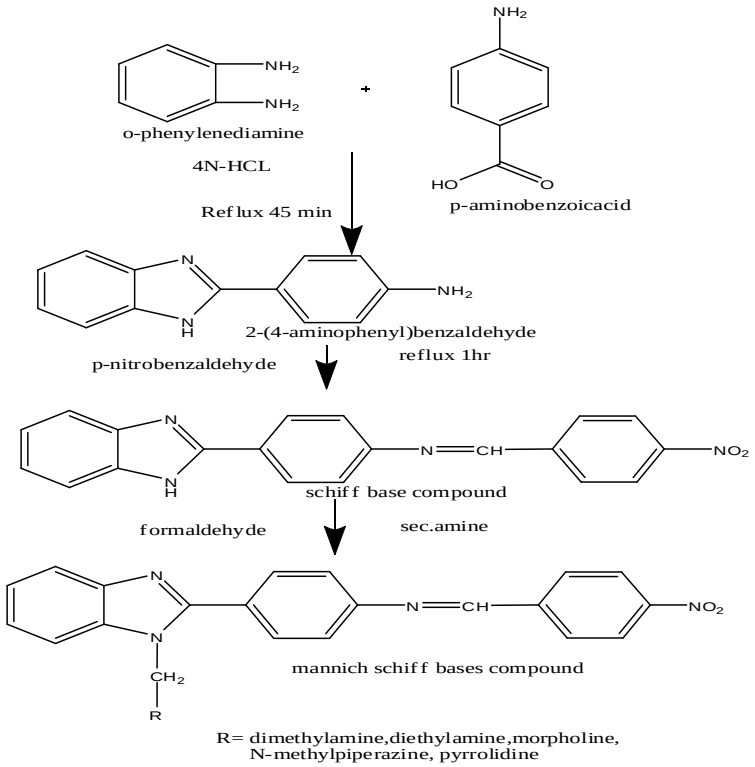
This compound was obtained as a pale yellow solid; Yield =88%, mp 270^{0}C-271^{0}C, Rf =0.71, IR (KBr) cm^{-1}: 3404.26, 3023.08, 2849.06, 1709.05, 1604.46, 1281.02, 851.05, 769.41;NMR($CDCl_3$): 8.4(3H,t,J=8.417), 8.1(3H,t,J=8.102), 7.3(1H,s,J=7.277), 1.4(1H,s,J=1.443); Mass: m/z 411.22 $C_{24}H_{21}N_5O_2$ (412.36): Calcd: C=70.03%; H=5.10%; N=17.02%; O=7.78%; Found: C=69.84%; H=5.09%; N=16.97%; O=7.76%.

Pharmacological Studies

Acute oral toxicity study

Acute oral toxicity studies were performed based on Organization of Economical Co-operation and Development (OECD) guidelines, for the synthesized compounds. Death was observed for synthesized compounds on administration of a dose of 2000mg/kg. Hence on the basis of this observation, a dose of 200mg/kg was selected for the anti-inflammatory studies.

Scheme-1:



Anti-inflammatory Studies

The anti-inflammatory activity was evaluated by Carrageenan induced rat paw edema method. Swiss albino Mice and Wister Rats weighing 30gm-250gm of either sex were divided into six animals in each groups. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% Carragenan in normal saline in the right hind paw of the rats, 1h after the administration of the drug. The paw diameter was measured by using digital varneir calipers at the intervals of 1 and 3 hrs after the Carrageenan injection. Indomethacin (10mg/kg, orally) was used as standard drug. The anti-inflammatory activity was calculated as percentage inhibition of Carrageenan induced paw edema using the following formula:

Percentage inhibition =
1-[(paw diameter in treated/paw diameter in control)×100]

Followed by Dunnet's test and result were reported to be statistically significant at $p<0.05$.

Table 1: Anti-inflammatory activity of the compounds

S. No.	Compounds	Dose (Mg/kg)	% Inhibition at 5hrs
1	BG1	200	69.44
2	BG2	200	68.27
3	BG3	200	76.28
4	BG4	200	65.44
5	BG5	200	71.13
6	Indomethacine (standard)	10	83.12

Antimicrobial activity

The antibacterial activity of the synthesized compounds was tested against gram (+)bacteria *Bacillus subtilis* (ATCC11774), *Staphylococcus aureus* (ATCC11632) and gram (-) bacteria *Escherichia coli* (ATCC10536)and *Klebsiella pneumonia*(ATCC10031) using Muller Hinton Agar medium.

Agar Well Diffusion Method

Petri plates containing 20ml Muller Hinton Medium were seeded with 24h culture of bacterial strains. Wells were cut and 20µl of the sample (of different concentrations) were added. The plates were then incubated at 37^0C for 24 hours. The antibacterial activities was assayed by measuring the diameter of the inhibition zone formed around the well. Ofloxacin were used as a positive control.

Table 2: Antimicrobial activity of Mannich Schiff base derivatives Zone of inhibition(mm)

SN	*B.subtilis*			*E.coli*			*S.aureus*			*K.pneumonia*		
Strain(mg/ml)	50	100	150	50	100	150	50	100	150	50	100	150
BG1	14	20	22	18	21	27	15	22	24	17	22	24
BG2	15	20	21	16	19	26	17	21	23	16	22	25
BG3	19	24	28	20	23	29	19	23	26	19	25	27
BG4	18	23	25	19	20	21	17	19	21	18	19	21
BG5	16	21	24	18	20	22	16	20	24	18	20	23
Ofloxacin(10mg/ml)	31			33			29			28		

RESULT AND DISCUSSION

2-(4-aminophenyl)benzimidazole was treated with p-nitrobenzaldehyde (aromatic aldehyde) to produce Schiff base. The Schiff base were subjected to Mannich reaction with formaldehyde and different secondary amines to produce Mannich Schiff base derivatives. The chemical structure of the synthesized compounds were confirmed by IR, ^{1}H-NMR, Mass spectral and elemental analysis.

The synthesized compounds were screened for antibacterial *Bacillus subtilis , Staphylococcus aureus , Escherichia coli* and *Klebsiella pneumonia* and anti-inflammatory activity by Carrageenan induced paw edema method. The antibacterial activity of all of the compounds showed lower potencies than the control drug oflaxacin by Agar Well Diffusion method. Compound BG3 showed good activity than others. All the synthesized compounds showed significant anti-inflammatory activity by Carrageenan induced paw edema method. Compound BG3 and BG5 showed better anti-inflammatory activity than other compounds.

CONCLUSION

The present work synthesized Mannich Schiff bases of substituted Benzimidazole and their derivatives. Five derivatives were prepared and biologically evaluated for antibacterial and anti-inflammatory activity. Nevertheless, some of the compounds were found to possess significant anti-inflammatory. Compound BG3 showed good antibacterial activity than other compounds. Therefore they may be used as lead compounds for further development.

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